

CLAIMS:

1. A method for the identification of T-cell stimulating protein fragments comprising the following steps:
 - a) establishing the amino acid sequence of an antigen which is a protein or a peptide;
 - b) subdividing the detected amino acid sequence of said antigen into protein fragments;
 - c) synthesizing at least one protein fragment having a length of from 8 to 30 amino acids, or cleaving the amino acid sequence of said antigen into at least one protein fragment having a length of from 8 to 30 amino acids, wherein said protein fragment is a subsequence of the established amino acid sequence of said antigen;
 - d) incubating a suspension containing T cells with the protein fragment or fragments in different experimental runs;
 - e) identifying of
 - (i) at least one T cell cytokin which has been induced by the protein fragment or fragments and synthesized in the T cells, wherein the T cell cytokin or cytokins remain within the cell or are bound to the cell membrane; and/or
 - (ii) at least one activation marker which has been induced or expression-enhanced by the protein fragment or

wherein said T cell cytokin or cytokins or activation markers are identified by flow cytometry; and

- f) assigning the experimental runs in which T cells have been stimulated and such stimulation has been recognized by the identification of one or more T cell cytokins and/or one or more activation markers, to the amino acid sequence or sequences of said protein fragments which had been incubated with the T cells.
2. The method for the identification of T-cell stimulating protein fragments according to claim 1, wherein said identification of at least one T cell cytokin or activation marker is made on the individual cell level.
3. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspensions containing T cells contain cells which present the protein fragment essentially in a state bound to MHC class I or class II molecules.
4. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the protein fragment in the class I restricted presentation comprises from 9 to 11 amino acids, and the protein fragment in the class II restricted presentation comprises at least 11 amino acids.

5. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspension containing T cells is a suspension of whole blood, peripheral white blood cells (PWBC), splenocytes, thymocytes, bone marrow, cerebrospinal fluid and/or lymph node cells.
6. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspension containing T cells is derived from the patients to be subjected to therapy, from donors or from animals.
7. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the antigens, i.e., proteins or peptides, are derived from macroorganisms, cells, cell cultures and/or tissues of donors or patients.
8. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the T cell cytokins are of the types interferon- γ , TNF- α or interleukin 2.
9. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the T cell cytokins remain within the cell after inhibition of secretion.
10. A process for the preparation of a protein fragment/peptide which is T-cell stimulating and whose amino acid sequence or initial amino acid sequence was found by the method for the identification of T-cell stimulating protein fragments according to any of the preceding claims 1 to 9, wherein said protein fragment/peptide is prepared by the solid phase method, liquid phase method or by protein biosynthesis in a host.

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11. The process for the preparation of a protein fragment/peptide according to claim 10, wherein said protein fragment/peptide contains insertions, deletions or substitutions (modifications) wherein one, two, three or more amino acids have been exchanged, deleted or inserted, wherein said modified protein fragment/peptide has essentially the same function with respect to the stimulation of T cells as the non-modified protein fragment/peptide.
12. The process for the preparation of a protein fragment/peptide according to claim 10 or 11, wherein said protein fragment/peptide contains at least one additional naturally occurring or not naturally occurring amino acid and/or a protecting group at the N-terminal and/or C-terminal end (extended modification), wherein the extendedly modified protein fragment/peptide has essentially the same function with respect to the stimulation of T cells as the non-modified protein fragment/ peptide.
13. Use of a protein fragment/peptide prepared by the process according to any of the preceding claims 10 to 12 for the preparation of a medicament for immune stimulation.
14. The use of a protein fragment/peptide according to claim 13, wherein said immune stimulation is a vaccination or desensitization.

method for the identification of T-cell stimulators comprising the following steps:

- i) establishing the amino acid sequence of said protein or a peptide;
- ii) subdividing the detected amino acid sequence into protein fragments;
- iii) synthesizing at least one protein fragment of from 8 to 30 amino acids, or cleaving the protein of said antigen into at least one protein fragment of from 8 to 30 amino acids, wherein said fragment is a subsequence of the amino acid sequence of said antigen;
- iv) incubating a suspension containing T cells with said fragment or fragments in different experiments;
- v) identifying of:
 - i) at least one T cell cytokin which has been released by the protein fragment or fragments and the cells, wherein the T cell cytokin or cytokins are released by the cell or are bound to the cell membrane;
 - ii) at least one activation marker expression which is enhanced due to the T cell stimulation by the protein fragment or fragments which has been released by the cell or is bound to the cell membrane;

1. A method for the identification of T-cell stimulating protein fragments comprising the following steps:
 - a) establishing the amino acid sequence of an antigen which is a protein or a peptide;
 - b) subdividing the detected amino acid sequence of said antigen into protein fragments;
 - c) synthesizing at least one protein fragment having a length of from 8 to 30 amino acids, or cleaving the amino acid sequence of said antigen into at least one protein fragment having a length of from 8 to 30 amino acids, wherein said protein fragment is a subsequence of the established amino acid sequence of said antigen;
 - d) incubating a suspension containing T cells with the protein fragment or fragments in different experimental runs;
 - e) identifying of
 - (i) at least one T cell cytokin which has been induced by the protein fragment or fragments and synthesized in the T cells, wherein the T cell cytokin or cytokins remain within the cell or are bound to the cell membrane; and/or
 - (ii) at least one activation marker expressed or expression-enhanced due to the T cell stimulation by the protein fragment or fragments which has been induced or

expression-enhanced by the protein fragment or fragments and which is expressed in the T cells, wherein said activation marker can be present within the cell or expressed on the cellular surface;

wherein said T cell cytokin or cytokins or activation markers are identified by flow cytometry; and

- f) assigning the experimental runs in which T cells have been stimulated and such stimulation has been recognized by the identification of one or more T cell cytokins and/or one or more activation markers, to the amino acid sequence or sequences of said protein fragments which had been incubated with the T cells;

characterized in that the incubation time is sufficiently long so that the protein fragment or fragments are sufficiently taken up by the MHC molecules present on the cellular surface, said taking up being sufficient when an unambiguous identification of stimulated T cells is possible; and

the incubation time of the suspension containing T cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T cells do not occur.

2. The method for the identification of T-cell stimulating protein fragments according to claim 1, wherein said identification of at least one T cell cytokin or activation marker is made on the individual cell level.

3. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspensions containing T cells contain cells which present the protein fragment essentially in a state bound to MHC class I or class II molecules.
4. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the protein fragment in the class I restricted presentation comprises from 9 to 11 amino acids, and the protein fragment in the class II restricted presentation comprises at least 11 amino acids.
5. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspension containing T cells is a suspension of whole blood, peripheral white blood cells (PWBC), splenocytes, thymocytes, bone marrow, cerebrospinal fluid and/or lymph node cells.
6. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein said suspension containing T cells is derived from the patients to be subjected to therapy, from donors or from animals.
7. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the antigens, i.e., proteins or peptides, are derived from polycellular eukaryotes, cells, cell cultures and/or tissues of donors or patients.
8. The method for the identification of T-cell stimulating protein fragments according to any of the preceding claims, wherein the T cell cytokins are of the types interferon- γ , TNF- α or interleukin 2.

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9. A process for the preparation of a protein fragment/peptide which is T-cell stimulating and whose amino acid sequence or initial amino acid sequence was found by the method for the identification of T-cell stimulating protein fragments according to any of the preceding claims 1 to 8, wherein said protein fragment/peptide is prepared by the solid phase method, liquid phase method or by protein biosynthesis in a host.
 10. The process for the preparation of a protein fragment/peptide according to claim 9, wherein said protein fragment/peptide contains insertions, deletions or substitutions (modifications) wherein one, two, three or more amino acids have been exchanged, deleted or inserted, wherein said modified protein fragment/peptide has essentially the same function with respect to the stimulation of T cells as the non-modified protein fragment/peptide.
 11. The process for the preparation of a protein fragment/peptide according to claim 9 or 10, wherein said protein fragment/peptide contains at least one additional naturally occurring or not naturally occurring amino acid and/or a protecting group at the N-terminal and/or C-terminal end (extended modification), wherein the extendedly modified protein fragment/peptide has essentially the same function with respect to the stimulation of T cells as the non-modified protein fragment/ peptide.
 12. Use of a protein fragment/peptide prepared by the process according to any of the preceding claims 9 to 11 for the preparation of a medicament for immune stimulation.
 13. The use of a protein fragment/peptide according to claim 12, wherein said immune stimulation is a vaccination or desensitization.

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